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DETECTION OF RICE WEEVIL LIFE STAGES IN RICE SEEDS USING RADIOGRAPHIC TECHNIQUES

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Abstract

Radiographic analysis is a nondestructive technique used to analyze the quality of seed lots and has shown efficiency and precision by reducing subjectivity and time in the performance of laboratory tests. The objective of this study was detect the developmental stages of S. oryzae (Coleoptera: Curculionidae) using X-rays. Healthy seeds of the rice cultivar CMG-1590 were infested with S. oryzae adults and evaluated by X-ray analysis according to the times estimated for the development of the stages weevil, namely, 5, 10, 20, 30 and 40 days after infestation. Additionally, analysis of the infested seeds were performed to evaluate the different life stages of the S. oryzae present. Statistical differences were observed regarding the levels of infestation detected between treatments. The egg, larva and adult stages of development were detected, except for the pupal stage, which was not possible to observe. Damaged seeds and empty seeds were also detected as a result of the feeding habit and life cycle of the insect. In conclusion, the X-ray analysis allowed us to verify the infestation of S. oryzae in rice seeds, as well as characterize the development cycle of the weevil and identify its life stages.

Key words**:** *seed infestation, sanitary quality, storage, X-rays*

INTRODUCTION

Rice is one of the most important commodities, is the third most common grain in the world, and is considered an energy and protein source with the ability to meet the daily caloric needs of millions of people worldwide [7], [15].

Productive success in rice fields involves the use of steps such as seed selection; sowing; the integrated management of pests, diseases and weeds implantation; harvest and post-harvest processing; harvesting and postharvest processing. The choice of rice seeds is very important because it is through them that the transfer of genetic advances of regional adaptability occurs, with the ability to provide better stand quality, plant vigor, initial development, plant uniformity in the field and increased productivity [17], [37]. Thus, rice seeds with high physical, physiological, genetic and health standards are considered essential inputs for the establishment and development of successful crops [12]. Among these attributes, sanitary quality is more relevant, as pests and diseases are usually spread by seeds [9], [25]. The examination of infested seeds is of paramount importance in the detection of storage insect pests, which can cause great economic losses in seed lots [9]. The beetles *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae), the weevils *Sitophilus oryzae* (Linnaeus, 1763) and *Sitophilus zeamais* (Montschulsky, 1885) (Coleoptera: Curculionidae) are considered primary and cross-infested pests and are capable of causing damage to seeds, both in the larval and adult stages. They can attack healthy seeds, pierce their external parts and consume their interiors to complete their life cycle [2], [29].

The detection and location of these pests in the early stages of development are difficult tasks; however, the use of methodologies that allow for the accurate and rapid identification of their presence is being increasingly studied [16]. Among the nondestructive techniques, the X-ray technique has been used in the evaluation of seed quality, determination of embryonic defects, morphological and morphometric evaluations, identification of broken seeds, and identification of seeds damaged by storage pests, bacterial and fungal infections [6], [33]. In the detection of stored seed pests, X-ray analysis has shown high repeatability and reproducibility in the determination of R. dominica infestation in wheat [20]. X-ray analysis has also been applied to analyze *Callosobruchus maculatus* (Fabricius, 1775) (Coleopetra: Bruchidae) on soybeans [8] and *S. zeamais* on maize seeds [14]. However, studies on the identification and verification of the developmental stages of this insect in stored rice seeds are still emerging [24], [35], [5]. Hence the need to research and develop new approaches that allow for the early and accurate detection of insect developmental stages, as well as the internal damage caused to rice seeds, offering a promising solution for assessing seed health, which may in the future contribute to the development of more efficient management and control strategies in storage.

Thus, the objective of the present study was to detect the developmental stages of *S. oryzae* (Coleoptera: Curculionidae) using X-rays

MATERIALS AND METHODS

This study was conducted at the Central Laboratory for Research on Seeds (CLRS) of the Department of Agriculture and the Laboratory of Ecotoxicology and Integrated Pest Management (LEMIP) of the Department of Entomology of the Federal University of Lavras (UFLA), in February and April 2022. Untreated rice seeds (CMG 1590 2020/2021 crop) developed by the Program for Genetic Improvement of Rice of the Highlands – “MelhorArroz da UFLA” in partnership with Empresa Brasileira de Pesquisa Agropecuaria rice and beans, and Empresa de Pesquisa Agropecuária de Minas Gerais were used. These seeds were infested with adult *S. oryzae* insects from the LEMIP laboratory.

To simulate infestation and ensure progression of *S. oryzae* developmental stages, five plastic containers with a capacity of 100 mL were used, each containing 40 g of rice seeds and 50 adult *S. oryzae* insects (aged 1-30 days). Seeds were collected at intervals of 5, 10, 20, 30, and 40 days after infestation, thus five treatments were defined based on the collection time after the initial infestation: T1 – seeds collected five days after infestation, corresponding to the egg stage; T2 – seeds collected to 10 days after infestation, corresponding to instars first and second of the larval stage; T3 – seeds to collected 20 days after infestation, corresponding to instars three and four of the larval stage; T4 – seeds collected 30 days after infestation, corresponding to the pupal stage; and T5 – seeds collected 40 days after infestation, corresponding to the presence of the pre-emergence adult stage in the rice seeds.

The containers were sealed with voile fabric to allow aeration, ensure survival, and prevent insect escape. The recipients were kept in climatic chambers at 25 ± 2°C and scotophase for 24 hours. The experimental design used was completely randomized, with five treatments and five replicates of 100 seeds of rice.

Sample collection and X-ray analysis

One hundred seeds were randomly removed from each container, constituting a replicate, for a total of 500 seeds evaluated for each treatment. For X-ray analysis, 100 seeds from each replicate were placed on acetate papers of 100 µm and fixed with double-sided transparent adhesive tape for fixation and subsequent individual identification.

Radiographic images were obtained using digital FAXITRON X-ray equipment, model MX-20, with a voltage adjustment of 30 kV and exposure for 19 seconds at a distance of 22.0 cm from the radiation source. Seeds with possible weevil presence at any developmental stage were set aside for further radiographic analysis, adjusting the focal length to 15 cm for clearer imaging while maintaining radiation intensity. Additionally, the radiographic images were used to determine the oviposition site in all infested rice seeds. Subsequently, the collected seeds were analyzed according to the methodology described in the Rules for Seed Analysis [4] to verify the presence or absence of each stage of development of the insect pest. For this purpose, the collected seeds were placed individually in plastic boxes and immersed in distilled water for a period of 24 hours. Afterwards, they were sectioned with a scalpel to evaluate the presence of eggs, larvae, pupae, adult insects or the exit holes of the insects.

Experimental design and statistical analysis

The infestation data were subjected to analysis of normality (Shapiro–Wilk; $p>0.05$) and homogeneity of variance (Bartlett; $p>0.05$). The F test of analysis of variance was performed, and the means were compared using Tukey's test ($p<0.05$). Data on the number of eggs, larvae, and adults and the detection of damaged and empty seeds were analyzed using the Kruskal–Wallis test, followed by the Bonferroni correction to determine differences between treatments ($p<0.05$). All analyses were performed with the Agricolae statistical package using R statistical software, version 4.1.1.

RESULTS AND DISCUSSIONS

Detection and characterization of rice weevil damage to rice seeds

Radiographic analyses of the rice seeds allowed for the detection and identification of the developmental stages of the weevil *S. oryzae* and the damage generated in the seeds (Figure 1). Radiographic images are characterized by white and black colors, where the color intensity is determined by the density and composition of the seed [20], [30]. Compared to the healthy seeds (Figure 1A), the infested seeds showed changes in internal tissues, especially in the endosperm, which is associated with the detection of the early stages of weevil development (Figure 1B, 1C and 1D) and with damaged and empty seeds (Figure 1E and 1F). Pupae were not detected by X-ray, and the absence of this stage was confirmed by traditional tests of infested seed analysis. X-ray analysis allowed the detection and identification of the infestation and developmental stages of *S. oryzae*, as well as the variations in the tissues that compose the seed, mainly due to the ability of the technique to detect hidden defects in the seeds [1]. The routine application of this technique in laboratories has shown high repeatability and reproducibility; however, its use still depends on the training of technicians to avoid misinterpretation of the images and on the acquisition of equipment, which has is expensive [14].

The oviposition hole detected in the X-ray test appeared as a dark circular or oval-shaped cavity located on the seed surface, with a small white spot (Figure 1B). Radiographic images of the oviposition site revealed 94.5% of the eggs in the endosperm and 5.6% in the perimeter of the rice seed embryo. Although the site of oviposition in the seed is not very clear, radiographic analyses conducted of wheat seeds infested with *S. oryzae* and found that the eggs are mostly present in the endosperm (69%) and in the perimeter of the embryo (11%) [32]. Through the observation of *Sitophilus* sp. in wheat [19] and maize [6] seeds via the X-ray technique, it was possible to distinguish oval-shaped and black sites that are related to the oviposition process. The female creates holes with her mandible, which is located at the distal end of her rostrum, and then secretes a gelatinous protective coating substance at the laying site [31], [10]. Internal detection in insect seeds at early stages of infestation tends to be confusing and difficult to interpret [8]. This is mainly due to the similarity between oviposition, larval instars and initial damage, which can be observed as small, darker gray spots. In addition, there are factors that make the distinction even more difficult, such as the density of the materials, the focal length [21] and the use or absence of contrasting agents, which can affect the quality of the image obtained [6], [14].

The shape and color of the larvae were detected, as they were oval and white and were present between the galleries and the seed tissues. These galleries or tunnels are formed as a function of the feeding habit of the larvae and were distinguished in the images as areas of darker color that contrasted with the endosperm of the seed (Figure 1C).

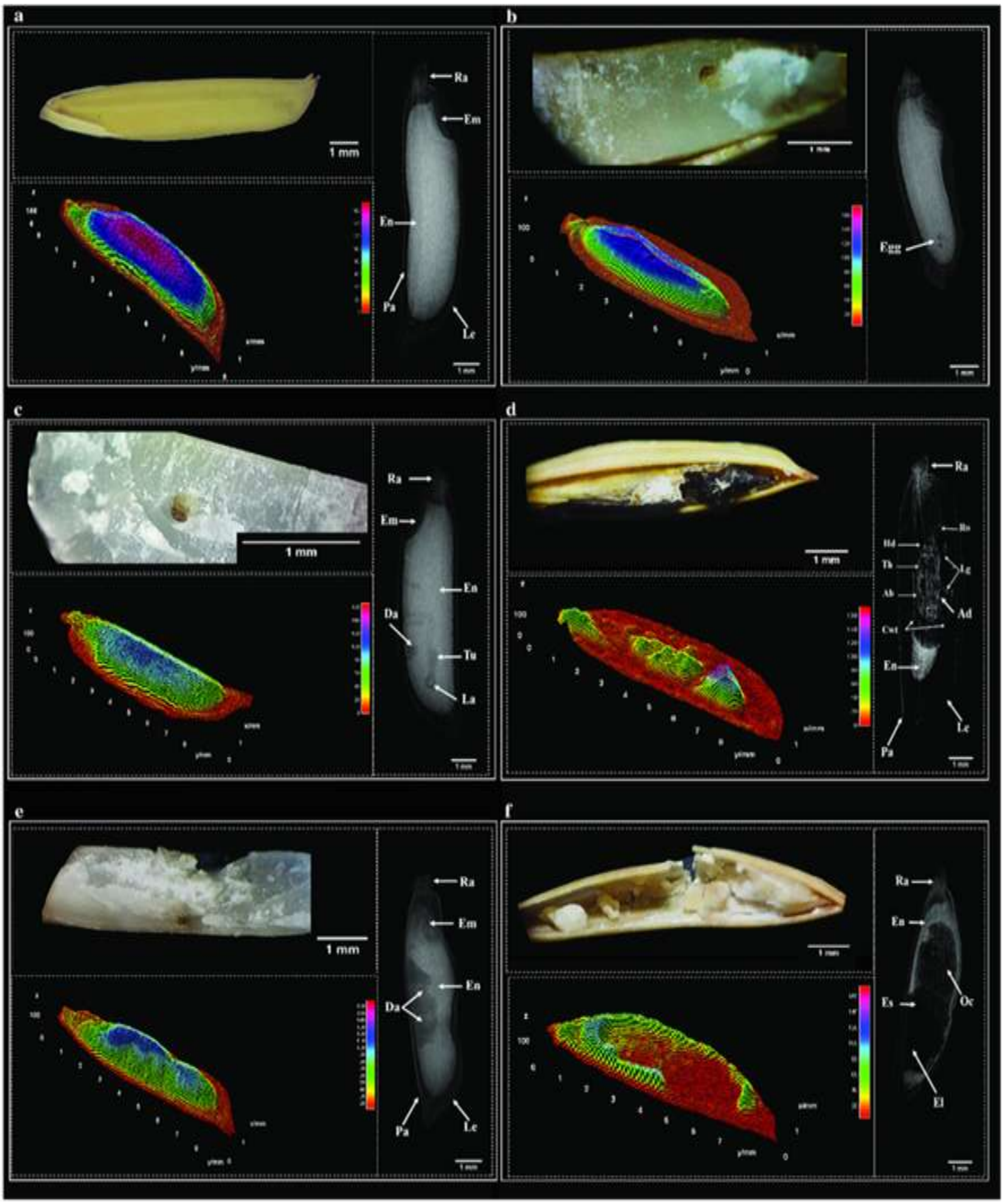

Fig 1. Detection of the developmental stages and characterization of *Sitophilus oryzae* damage on rice seeds by examining infested seeds and X-rays: A) healthy seed; B) egg; C) larva (La) and tunnel (Tu); D) Adult (Ad); E) Damage seed (Da); F) Empty seed (Es). Seed structure: embryo (Em), endosperm (En), caryopsis wrap tissue (Cwt), rachilla (Ra), lemma (Le), palea (Pa), oval cavity (Oc) and exit location (El). Adult parts inside the seed: abdomen (Ab), head (Hd), rostrum (Ro), thoracic segment (Th) and Leg (Lg).
Source: Authors' own illustration.

Approximately five days after oviposition, the larvae hatch, begin feeding on the endosperm and pass through four larval instars until they pupate [10], [3]. This characteristic makes identification easier in more advanced instars due to the size of the larval body [18]. Research shows that after 20 days of infestation with *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Curculuonidae) in wheat seeds, galleries and voids, which tend to be darker in color, were the dominant features in X-ray images [13], [26]. This contrasts with the white colors of the tissues not affected by the larvae, [8], [14], [5]. In the last instar, a dark oval cavity forms [6], [19], where pupal formation occurs [18].

Radiographically, preemergent adults tend to be distinguished by the clear identification of certain anatomical structures that have already developed in the body of the insect, such as the abdomen, rostrum and legs of the insect. The adults were observed inside a cavity in the tissues of the caryopsis envelope, protected by the seed shell, which contrasted by presenting dark gray colors compared to the white portions of the endosperm not consumed during the larval stage (Figure 1D). When the adult emerges, it remains inside the seed, moving to find the thinnest place to consume and perforate the seed tissues (envelope of the caryopsis, palea or lemma) and thus creates at hole where it exits the seed [32], [11].

After the emergence of the adult, empty tunnels located in wheat [6], [19] and maize seeds [34] were observed in radiographic images, which presented lower density in the tissues than healthy grains or with than grains with larvae inside them [5].

The damage caused by the rice weevil is mainly the result of the feeding habit of this pest and is differentiated according to the amount of tissue affected in the seed, which begins in the external tissues of the seeds, such as the palea, lemma and caryopsis envelope, until reaching the endosperm. The damage was also distinguished by dark rounded spots that contrasted with the light tones of the endosperm and seed embryo, although in advanced damage, irregular spots of larger size were observed that affected a large part of the endosperm (Figure 1E).

The empty seeds are the final result of the developmental cycle of the rice weevil, with the emergence of the adult insect, since consumption of a large part of the endosperm occurred for its development, forming a cavity, usually oval, with attenuations that contrasted with residues of the endosperm and embryo, and with the healthy seeds. It was also possible to identify the weevil emergence site, which was characterized by rupture of the pericarp and the palea and lemma (Figure 1F).

Percent infestation and detection of weevil developmental stages

Data analysis using a box plot (Figure 2) indicated that the infestation had a normal distribution for the evaluated treatments, in addition to the absence of nonstandard data. The percentages of infestation detected by X-rays at 5, 10, 20, 30 and 40 days after infestation ranged from 0.0% to 2.0%, 2.0% to 4.0%, 1.0% to 4.0%, 1.0% to 4.0%, and 4.0% to 8.0%, respectively (Figure 2).

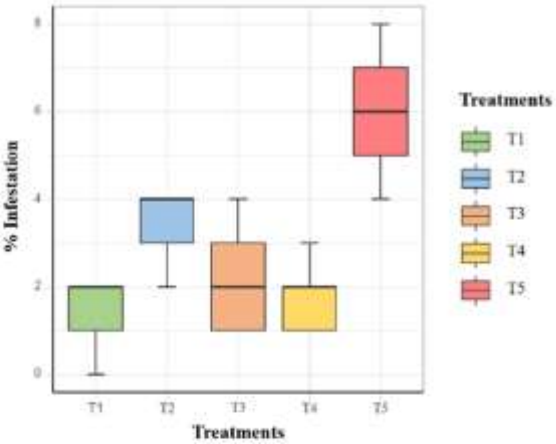

Fig 2. Box plot for the percentage of *Sitophilus oryzae* infestation detected by X-rays. The box plots show the median plus the upper and lower quartiles of the percentage of infestation for each treatment. The minimum and maximum values are indicated by the lower and upper limits, respectively. Treatments were evaluated at 5(T1), 10 (T2), 20 (T3), 30 (T4) and 40 (T5) days after infestation.
Source: Authors' determination.

The percentages of infestation detected showed significant differences ($p < 0.05$), where the T5 treatment presented the highest level of infestation (Figure 3), which may be related to the longer time needed to complete its biological cycle and ensure greater numbers of offspring.

The percentage of eggs identified and the percentage of damaged seeds showed no significant differences between the infestation times according to the Kruskal–Wallis test ($p > 0.05$).

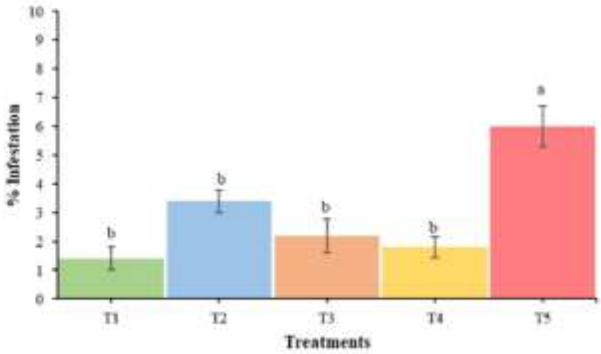

Fig 3. Percentages of infestation detected by the radiographic images at the sampling times. The different letters above the columns for each treatment indicate significant differences according to Tukey's test ($p < 0.05$). The bars represent the standard error. Treatments evaluated at 5 (T1), 10 (T2), 20 (T3), 30 (T4) and 40 (T5) days after infestation of rice seeds by *Sitophilus oryzae.*
Source: Authors' determination.

The percentages of larvae, adults and empty seeds detected by X-ray images and confirmed by the infested seeds test were significantly greater in treatment T5, which corresponded to the longer exposure time to infestation with *S. oryzae* (Table 1).

The traditional analysis of infested seeds has been shown to be susceptible to human error, high subjectivity and slowness when evaluating large amounts of seeds and even more when they are smaller [36].

This justifies the importance of using more precise tools and techniques, such as X-ray analysis, for the detection of insect pests inside seeds. As insects are detected at more advanced stages of development, a greater amount of affected tissue is observed [26], [27], [5]. Thus, image analysis tools could assist in the detection and quantification of the effects of seed storage pests, and thus establish models that aid in the quality control of seed batches [34], [22].

This analytical technique has demonstrated potential in determining the percentage of infestation, as well as advances in the evaluation of the sanitary quality of seeds, aiming to safeguard the quality of seed lots.

Table 1. Differences in the mean percentage ± SE in the detection of developmental stages and the identification of damaged of *Sitophilus oryzae* in rice seeds.

Treatment	Developmental stages			Damage seeds	
	% egg	% Larva	% Adult*	% Damage	% Empty
T1	1.0±0.32a	0.0±0.0b	0.0±0.00b	0.4±0.25a	0.0±0.00b
T2	1.6±0.24a	0.4±0.25b	0.0±0.00b	1.4±0.60a	0.0±0.00b
T3	0.8±0.20a	0.0±0.0b	0.0±0.00b	1.4±0.60a	0.0±0.00b
T4	1.0±0.32a	0.4±0.25b	0.0±0.00b	0.4±0.25a	0.0±0.00b
T5	0.8±0.20a	2.0±0.54a	1.4±0.60a	0.8±0.20a	1.0±0.32a

Note: Means in the column followed by the same letter are not significantly different ($p > 0.05$) according to the Kruskal–Wallis test. Treatments evaluated at 5 (T1), 10 (T2), 20 (T3), 30 (T4) and 40 (T5) days after seed infestation by the rice weevil. *Preemerged adult. SE: Standard error.
Source: Authors' determination.

This approach can also reduce the time required to obtain results, allowing the detection of insects in a few seconds or minutes [23], [28], in addition to being repeatable, reliable and easy to perform results, compared to traditional infested seed tests, in which it is necessary to soak the seeds for 24 hours before being segmented and to perform visual confirmation of the presence or absence of insect pests [4].

CONCLUSIONS

The use of X-ray analysis allows for the characterization and identification of *S. oryzae* at the egg, larval and adult stages in rice seeds. The damage caused by the larvae and adults of *S. oryzae* in rice seeds is characterized by the formation of tunnels and consumption of the endosperm and embryo. The empty rice seeds and endosperm traces observed via X-ray analysis are the result of the completion of the weevil development cycle, which culminates with the exit of the adult insect from the seed.

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